(For Laboratory Use Only) CLH 4/22/16
Accuratus Lab Services Project # A20729
Test Substance Tracking # TS041516.RUOOL
KBH 4-18-16

ACCURATUS LAB SERVICES

PROTOCOL

EPA Hard Surface Mildew-Fungistatic Test

Test Organism:

Aspergillus niger (ATCC 6275)

PROTOCOL NUMBER

RUO01040416.MSTAT

PREPARED FOR

Rust-Oleum
11 Hawthorne Parkway
Vernon Hills, IL 60061

SPONSOR REPRESENTATIVE

Haley & Aldrich
455 E. Eisenhower Pkwy, Suite 210
Ann Arbor, MI 48108-3323

PERFORMING LABORATORY

Accuratus Lab Services
1285 Corporate Center Drive, Suite 110
Eagan, MN 55121

DATE

April 4, 2016

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EXACT COPY
INITIALS JLH DATE 5-24-16

EPA Hard Surface Mildew-Fungistatic Test

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Vernon Hills, IL 60061

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TEST FACILITY: Accuratus Lab Services
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PURPOSE
The purpose of this study is to demonstrate fungistatic effectiveness of products intended for use to control, prevent, or inhibit the growth of fungi which cause mildew on various hard surfaces.

TEST SUBSTANCE CHARACTERIZATION
According to (40 CFR, Part 160, Subpart F [160.105]) test substance characterization as to identity, strength, purity, solubility and composition, as applicable, shall be documented before its use in this study. The stability of the test substance shall be determined prior to or concurrently with this study. Pertinent information, which may affect the outcome of this study, shall be communicated in writing to the Study Director upon sample submission to Accuratus Lab Services. Accuratus Lab Services will append Sponsor-provided Certificates of Analysis (C of A) to this study report, if requested and supplied. Characterization and stability studies not performed following GLP regulations will be noted in the Good Laboratory Practice compliance statement.

SCHEDULING AND DISCLAIMER OF WARRANTY
Experimental start dates are generally scheduled on a first-come/first-serve basis once Accuratus Lab Services receives the Sponsor approved/completed protocol, signed fee schedule and corresponding test substance(s). Based on all required materials being received at this time, the proposed experimental start date is April 18, 2016. Verbal results may be given upon completion of the study with a written report to follow on the proposed completion date of May 16, 2016. To expedite scheduling, please be sure all required paperwork and test substance documentation is complete/accurate upon arrival at Accuratus Lab Services.

If a test must be repeated, or a portion of it, due to failure by Accuratus Lab Services to adhere to specified procedures, it will be repeated free of charge. If a test must be repeated, or a portion of it, due to failure of internal controls, it will be repeated free of charge. "Methods Development" fees shall be assessed, however, if the test substance and/or test system require modifications due to complexity and difficulty of testing.

If the Sponsor requests a repeat test, they will be charged for an additional test.

Neither the name of Accuratus Lab Services nor any of its employees are to be used in advertising or other promotion without written consent from Accuratus Lab Services.

The Sponsor is responsible for any rejection of the final report by the regulatory agencies concerning report format, pagination, etc. To prevent rejection, Sponsor should carefully review the Accuratus Lab Services final report and notify Accuratus Lab Services of any perceived deficiencies in these areas before submission of the report to the regulatory agency. Accuratus Lab Services will make reasonable changes deemed necessary by the Sponsor, without altering the technical data.

JUSTIFICATION FOR SELECTION OF THE TEST SYSTEM
The U.S. Environmental Protection Agency requires that a specific claim for a fungistat be supported by appropriate scientific data demonstrating the efficacy of the test substance against the claimed microorganism. This is accomplished, in the laboratory, by treating the target organism with the fungistat (test substance) under conditions which simulate as closely as possible the actual conditions under which the test substance is designed to be used. The test system to be used in this study will follow the EPA approved Hard Surface Mildew Fungistatic test method as specified in the EPA Pesticide Assessment Guidelines Subdivision G.

TEST PRINCIPLE

Sterile carriers are treated with the test substance and incubated until dry. The carriers are then sprayed with a spore suspension of a mildew causing organism and incubated at high humidity. Following incubation, the carriers are visually examined for the presence of test organism. Appropriate culture purity and sterility controls are performed. The current version of Standard Operating Procedure CGT-0042 reflects the methods which shall be used in this study.

TEST METHOD

Test Organism	ATCC #	Growth Medium	Incubation Parameters
Aspergillus niger	6275	Sabouraud agar (modified)	25-30°C, aerobic

The test organism used in this study was obtained from the American Type Culture Collection (ATCC), Manassas, Virginia.

Carriers

Glazed ceramic tiles: 1 inch x 1 inch carriers, sterilized for ≥ 2 hours at ≥180°C in hot air oven.

Preparation of Test Substance

The test substance(s) to be assayed will be used as directed by the Sponsor. If a dilution of the test substance is requested by the Sponsor, the diluted test substance(s) shall be used within three hours of preparation.

Test Substance Application

If the product is a spray application, the surfaces of 10 carriers will be sprayed as specified by the Sponsor.

If the product is a liquid application, the surfaces of 10 carriers will be treated by immersing the carriers in the test substance until completely covered and will be removed.

If the product is a towelette application, the glazed area of the carriers will be treated with the towelette in a motion described by the Sponsor.

Treated carriers will be placed in a vertical or near vertical position to permit excess liquid to drain. The carriers will be dried in Petri dishes at 35-37°C with lids ajar.

Control Carriers

Untreated carriers will be placed in sterile Petri dishes (10 carriers total) and dried at 35-37°C with lids ajar alongside the test carriers.

Preparation of Test Organism

The *Aspergillus niger* conidial suspension will be prepared by inoculating a flask of Sabouraud agar (Modified) *(aka Neopeptone agar)* and incubating for **7-10 days** at 25-30°C. Following incubation, sterile saline or saline/Triton Solution (0.85% Saline + 0.05% Triton X-100) and sterile glass beads are added to the flask. The flask is agitated to remove mycelia/conidia from the agar. The conidia suspension is aspirated from the flask and passed through sterile gauze to remove hyphal fragments. The conidial concentration is estimated by counting in a hemacytometer. The suspension will be added to a sterile tissue grinder and macerated to break up spore chains. This may be performed at the time of harvest or on the day of testing.

The macerated conidial suspension will be standardized to contain an approximate target of 5×10^6 conidia per mL. One (1.0) mL of this suspension is added to 20.0 mL of sterile Czapek's solution. An organic soil load may be added to the prepared Czapek/organism suspension per Sponsor's request.

Contamination of Carriers

Following the initial drying period, an atomizer will be used to spray the surface of each test carrier and control carrier with the *Aspergillus niger* conidia-Czapek suspension. The atomizer will be periodically shaken to agitate the culture during inoculation. Carriers contained in Petri dishes will be returned to 35-37°C incubator and dried with the lids slightly ajar until visibly dry. Excessive drying should be avoided. Typically, carriers will be dry after approximately 45 minutes of drying.

Incubation
Each carrier (sprayed side up) will be placed onto an individual water agar plate. All plates are incubated for 7 days at 25-30°C in a minimum of 95% relative humidity. (A desiccator containing approximately 100 mL of deionized water is recommended.) Refrigeration of test/control carriers is not allowed following incubation unless otherwise noted.

Evaluation
The purity control will be incubated for 44-76 hours at 25-30°C and may be refrigerated for up to 3 days at 2-8°C prior to examination. All test and control carriers will be examined after 7 days of incubation. The absence of fungal growth on all carriers is the criterion for determining the effectiveness of the test product. When no visual growth is evident at the end of the 7 days on test carriers, a magnified examination is performed. To be considered a valid test, each untreated control carrier must be at least 50% covered with fungal growth after the 7 days. At least one test carrier per test substance sample set showing growth will be confirmed as the test organism by Lactophenol cotton blue (tape) staining. Remaining test carriers demonstrating growth morphology consistent with the confirmed carrier will be considered confirmed as the test organism.

STUDY CONTROLS

Purity Control
A "streak plate for isolation" will be performed on the organism culture. Following incubation, the culture is examined in order to confirm the presence of a pure culture. The acceptance criterion for this study control is a pure culture demonstrating colony morphology typical of the test organism.

Carrier Sterility Control
A representative uninoculated carrier will be added to a water agar plate. The carrier will be incubated and examined for growth. The acceptance criterion for this study control is lack of growth.

Agar Sterility Control
A representative sample of uninoculated water agar will be incubated and visually examined. The acceptance criterion for this study control is lack of growth.

Organic Soil Load Sterility Control
If applicable, the serum used for soil load will be added to a tube of Fluid Thioglycollate Medium, incubated alongside the test, and visually examined for lack of growth. The acceptance criterion for this study control is lack of growth.

PROCEDURE FOR IDENTIFICATION OF THE TEST SYSTEM
Accuratus Lab Services maintains Standard Operating Procedures (SOPs) relative to efficacy testing studies. Efficacy testing is performed in strict adherence to these SOPs which have been constructed to cover all aspects of the work including, but not limited to, receipt, log-in, and tracking of biological reagents including test organism strains for purposes of identification, receipt and use of chemical reagents. These procedures are designed to document each step of efficacy testing studies. Appropriate references to medium, batch number, etc. are documented in the raw data collected during the course of each study.

Additionally, each efficacy test is assigned a unique Project Number when the protocol for the study is initiated by the Study Director. This number is used for identification of the test subcultures, etc. during the course of the test. Test subcultures are also labeled with reference to the test organism, experimental start date, and test product. Microscopic and/or macroscopic evaluations of positive subcultures are performed in order to confirm the identity of the test organism. These measures are designed to document the identity of the test system.

METHOD FOR CONTROL OF BIAS: NA

STUDY ACCEPTANCE CRITERIA

Test Substance Performance Criteria
The EPA efficacy performance requirements for label claims state that all treated replicates must be free of fungal growth.

Control Acceptance Criteria
The study controls must perform according to the criteria detailed in the evaluation section and the study controls description section. If any of the control acceptance criteria are not met, the test may be repeated under the current protocol number.

REPORT
The report will include, but not be limited to, identification of the sample, date received, initiation and completion dates, identification of the bacterial strains used, description of media and reagents, description of the methods employed, tabulated results and conclusion as it relates to the purpose of the test, and all other items required by 40 CFR Part 160.185.

PROTOCOL CHANGES
If it becomes necessary to make changes in the approved protocol, the revision and reasons for change will be documented, reported to the Sponsor and will become a part of the permanent file for that study. Similarly, the Sponsor will be notified as soon as possible whenever an event occurs that may have an effect on the validity of the study.

Standard operating procedures used in this study will be the current effective revision at the time of the work. Any minor changes to SOPs (for this study) or methods used will be documented in the raw data and approved by the Study Director.

TEST SUBSTANCE RETENTION
It is the responsibility of the Sponsor to retain a sample of the test substance. All unused test substance will be discarded following study completion unless otherwise indicated by Sponsor.

RECORD RETENTION

Study Specific Documents

All of the original raw data developed exclusively for this study shall be archived at Accuratus Lab Services for a minimum of five years for GLP studies or a minimum of six months for all other studies following the study completion date. After this time, the Sponsor (or the Sponsor Representative, if applicable) will be contacted to determine the final disposition. These original data include, but are not limited to, the following:

1. All handwritten raw data for control and test substances including, but not limited to, notebooks, data forms and calculations.
2. Any protocol amendments/deviation notifications.
3. All measured data used in formulating the final report.
4. Memoranda, specifications, and other study specific correspondence relating to interpretation and evaluation of data, other than those documents contained in the final study report.
5. Original signed protocol.
6. Certified copy of final study report.
7. Study-specific SOP deviations made during the study.

Facility Specific Documents

The following records shall also be archived at Accuratus Lab Services. These documents include, but are not limited to, the following:

1. SOPs which pertain to the study conducted.
2. Non study-specific SOP deviations made during the course of this study which may affect the results obtained during this study.
3. Methods which were used or referenced in the study conducted.
4. QA reports for each QA inspection with comments.
5. Facility Records: Temperature Logs (ambient, incubator, etc.), Instrument Logs, Calibration and Maintenance Records.
6. Current curriculum vitae, training records, and job descriptions for all personnel involved in the study.

REFERENCE

1. U.S. Environmental Protection Agency Pesticide Assessment Guidelines, Subdivision G: Product Performance, November, 1982, Section 93-30, I. Surfaces (Mold and Mildew), Item 2, Hard Surface Mildew Fungistatic Test Method.

DATA ANALYSIS

Calculations
None used.

Statistical Analysis
None used.

STUDY INFORMATION

(All blank sections are completed by the Sponsor or Sponsor Representative as linked to their signature, unless otherwise noted.)

Test Substance (Name and Batch/Lot Number - exactly as it should appear on final report):
(1) Mold Killing Primer Aerosol - Lot 1 dosed Book 600-075 (3) Aerosol Paint Control - Blank
(3) Mold Killing Primer Aerosol - Lot 2 dosed Book 600-077 Book 600-075

Product Description:
- ☐ Quaternary ammonia
- ☐ Iodophor
- ☐ Sodium hypochlorite
- ☐ Peracetic acid
- ☐ Peroxide
- ☒ Other 3-iodo 2 propynyl butyl carbamate (IPBC)

Approximate Test Substance Active Concentration (upon submission to Accuratus Lab Services):
0.135 %
(This value is used for study planning only. This value is not intended to represent characterization values.)

Storage Conditions
- ☒ Room Temperature
- ☐ 2-8°C
- ☐ Other_____

Hazards
- ☐ None known: Use Standard Precautions
- ☒ Material Safety Data Sheet, Attached for each product
- ☐ As Follows:_____

Product Preparation
- ☒ No dilution required, Use as received (RTU)
- ☐ *Dilution(s) to be tested:
  _____ defined as _____ + _____
  (example: 1 oz/gallon) (amount of test substance) (amount of diluent)
  - ☐ Deionized Water (Filter or Autoclave Sterilized)
  - ☐ Soft Tap Water (Filter or Autoclave Sterilized)
  - ☐ AOAC Synthetic Hard Water: _____PPM
  - ☒ Other Shake well before use

Note: An equivalent dilution may be made unless otherwise requested by the Sponsor.

Test Organism: *Aspergillus niger* (ATCC 6275)

Test Substance Application:
- ☒ **Spray application products:** One or until thoroughly wet ①
  Number of sprays per carrier: One or _____ or Spray time per carrier: _____ seconds
  Approximate Spray distance: 8-12"
- ☐ **Liquid Application: Immerse carriers in test substance**
- ☐ **Towelette application:** ☐ Typical: Each carrier will be treated by wiping the glazed area with the towelette over and back, _____ (#) times for a total of _____ (#) passes per surface. (i.e. over and back 2 times equals a total of 4 passes)
  ☐ Other: _____

Organic Soil Load:
- ☒ Minimum 5% Organic Soil Load (fetal bovine serum)
- ☐ No Organic Soil Load Required
- ☐ Other_____

① Spelling error. JLH 4-20-16

TEST SUBSTANCE SHIPMENT STATUS

(This section is for informational purposes only.)

☐ Test Substance is already present at Accuratus Lab Services.
☑ Test Substance has been or will be shipped to Accuratus Lab Services.
Date of expected receipt at Accuratus Lab Services: April 11, 2016
☐ Test Substance to be hand-delivered (must arrive by noon at least one day prior to testing or other arrangements made with the Study director)

COMPLIANCE

Study to be performed under EPA Good Laboratory Practice regulations (40 CFR Part 160) and in accordance to standard operating procedures.
☑ Yes
☐ No (Non-GLP or Development Study)

PROTOCOL MODIFICATIONS

☐ Approved without modification
☑ Approved with modification
In addition to the standard 2 lot test, one lot of control material will be tested using 3 test carriers. The results of the control lot will be for informational purposes only and will have no acceptance criteria.

PROTOCOL ATTACHMENTS

Supplemental Information Form Attached - ☐ Yes ☑ No

TEST SUBSTANCE CHARACTERIZATION & STABILITY TESTING
[Verification required per 40 CFR Part 160 Subpart B (160.31(d))].

☐ Characterization/Stability testing is not required (For Non-GLP or Development testing only)

OR

Physical and Chemical Characterization (identity, purity, strength, solubility, as applicable) of the test lots

☑ **Physical & Chemical Characterization has been or will be completed prior to efficacy testing.**

GLP compliance status of physical & chemical characterization testing:
☑ Testing was or will be performed following 40 CFR Part 160 GLP regulations
☐ Characterization has not been or will not be performed following GLP regulations

Check and complete the following that apply:
☐ A Certificate of Analysis (C of A) has been or will be provided for each lot of test substance to be appended to the report.
☐ Testing has been or will be conducted at Accuratus Lab Services under protocol or study #: _____
☐ Test has been or will be conducted by another facility under protocol or study #: _____

☐ **Physical & Chemical Characterization was not or will not be performed prior to efficacy testing.**

Stability Testing of the formulation

① ☒ **Stability testing has been or will be completed prior to or concurrent with efficacy testing.**

GLP compliance status of stability testing:
(GLP compliance is required by 40 CFR Part 160)
① ☒ Testing was or will be performed following 40 CFR Part 160 GLP regulations
☐ Stability testing has not been or will not be performed following GLP regulations

Check and complete the following that apply:
☐ Testing has been or will be conducted at Accuratus Lab Services under protocol or study #: _____
☐ Test has been or will be conducted by another facility under protocol or study #: Product Safety Labs Study # 42075 (dated 1/5/2016)

☐ **Stability testing was not or will not be performed prior to or concurrent with efficacy testing.**

If test substance characterization or stability testing information is not provided or is not performed following GLP regulations, this will be indicated in the GLP compliance statement of the final report.

① Added per email. JLH 4-19-16

APPROVAL SIGNATURES

SPONSOR:

NAME: Ms. Maryann Sanders TITLE: Agent

SIGNATURE: Maryann C. Sanders DATE: 4/15/2016

PHONE: ~~(248) 662-3920~~ 374-3957 FAX: (734) 877 - 8425 EMAIL: msanders@HaleyAldrich.com

For confidentiality purposes, study information will be released only to the sponsor/representative signing the protocol (above) unless other individuals are specifically authorized in writing to receive study information.

Other individuals authorized to receive information regarding this study: ☐ See Attached

Accuratus Lab Services:

NAME: Jamie Herzan
Study Director

SIGNATURE: Jamie Herzan DATE: 4-22-16
Study Director